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The arrangement shown in Figure 65a, in which the probe molecule is completely complementary to the target strand, is similar to that shown in Figure 32. Treatment of the top structure with the 5' nuclease of a DNA polymerase would cause exonucleolytic nibbling of the probe (*i.e.*, in the absence of the upstream oligonucleotide). In contrast, inclusion of an invader oligonucleotide would cause a distinctive cleavage shift similar, to those observed in Figure 33.

The arrangements shown in Figures 65b and 65c have some amount of unpaired sequence at the 5' terminus of the probe (3 and 5 bases, respectively). These small 5' arms are suitable cleavage substrate for the 5' nucleases and would be cleaved within 2 nucleotide's of the junction between the single stranded region and the duplex. In these arrangements, the 3' end of the upstream oligonucleotide shares identity with a portion of the 5' region of the probe which is complementary to the target sequence (that is the 3' end of the invader has to compete for binding to the target with a portion of the 5' end of the probe). Therefore, when the upstream oligonucleotide is included it is thought to mediate a shift in the site of cleavage into the downstream duplex (although the present invention is not limited to any particular mechanism of action), and this would, therefore, constitute invasive cleavage. If the extreme 5' nucleotides of the unpaired region of the probe were able to hybridize to the target strand, the cleavage site in the absence of the invader might change but the addition of the invader oligonucleotide would still shift the cleavage site to the proper position.

Finally, in the arrangement shown in Figure 65d, the probe and upstream oligonucleotides share no significant regions of homology, and the presence of the upstream oligonucleotide would not compete for binding to the target with the probe. Cleavage of the structures shown in Figure 64d would occur at the same site with or without the upstream oligonucleotide, and is thus would not constitute invasive cleavage.

By examining any upstream oligonucleotide/probe pair in this way, it can easily be determined whether the resulting cleavage is invasive or merely primer-directed. Such analysis is particularly useful when the probe is not fully complementary to the target nucleic acid, so that the expected result may not be obvious by simple inspection of the sequences.

From the above it is clear that the invention provides reagents and methods to permit the detection and characterization of nucleic acid sequences and variations in nucleic acid sequences. The invader-directed cleavage reaction of the present invention provides an ideal direct detection method that combines the advantages of the direct detection assays (*e.g.*, easy quantification and minimal risk of carry-over contamination) with the specificity provided by a dual oligonucleotide hybridization assay.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Prudent, James R.
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- (ii) TITLE OF INVENTION: Invasive Cleavage Of Nucleic Acids
- (iii) NUMBER OF SEQUENCES: 69
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- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2506 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)